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Formulation and Characterization of Herbal Topical Preparation for Wound Healing

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ABSTRACT
The present study focuses on the development and evaluation of a polyherbal topical gel formulation for wound healing activity using Plumbago zeylanica, Datura stramonium, and Argemone mexicana. Wound healing is a complex biological process involving inflammation, proliferation, and remodeling, often affected by microbial infection and oxidative stress. Herbal medicines, rich in bioactive phytoconstituents, offer a safer and effective alternative to synthetic drugs. In this study, plant extracts were prepared using Soxhlet extraction and evaluated for physicochemical properties, phytochemical constituents, antioxidant activity, and antimicrobial activity. The polyherbal formulation demonstrated the presence of alkaloids, flavonoids, tannins, glycosides, and other bioactive compounds. The antioxidant activity assessed by DPPH assay showed significant free radical scavenging activity, with the polyherbal extract exhibiting superior activity compared to individual extracts. Antimicrobial studies revealed effective inhibition against both gram-positive and gram-negative bacteria. The formulated gel showed acceptable physicochemical properties, stability, and sustained drug release. The study concludes that the developed polyherbal gel is a promising, safe, and effective formulation for wound healing applications.

Keywords
Polyherbal gel,
Wound healing,
Antioxidant activity,
Antimicrobial activity,
Plumbago zeylanica

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Introduction

Medicinal plants have been used since ancient times for the treatment of various diseases due to their rich content of bioactive compounds such as flavonoids, tannins, alkaloids, and phenolic compounds, which exhibit antimicrobial, antioxidant, and anti-inflammatory activities [1,2]. These phytoconstituents play a vital role in promoting wound healing by enhancing tissue regeneration and reducing microbial infections.

Wounds are defined as the disruption of normal anatomical and functional continuity of tissues caused by physical, chemical, or biological factors [3]. The wound healing process involves a series of complex biological events including inflammation, proliferation, and remodeling phases, which require coordinated cellular and molecular responses [4]. However, factors such as infection, poor nutrition, diabetes, and reduced oxygen supply can delay the healing process [5].

Conventional wound healing therapies often involve the use of antibiotics and synthetic drugs, which may cause side effects and lead to microbial resistance. Therefore, there is a growing interest in herbal formulations as safer and more effective alternatives [6]. Medicinal plants such as Plumbago zeylanica, Datura stramonium, and Argemone mexicana have been widely reported for their antimicrobial, antioxidant, and wound healing properties [7–9].

Topical drug delivery systems such as gels offer several advantages, including ease of application, improved drug penetration, and better patient compliance [10]. Herbal gels, in particular, provide a suitable medium for delivering plant-based bioactive compounds directly to the wound site. Therefore, the present study aims to develop and evaluate a polyherbal gel formulation incorporating selected medicinal plant extracts for enhanced wound healing activity, combining traditional knowledge with modern pharmaceutical approaches.

Materials and Methods

Collection and authentication of plant materials

Plumbago zeylanica linn, Datura stromonium L and Argemone Mexicana linn were collected from nearby areas of Nellore district, the plant materials were identified and authenticated by Dr. K. Madhava Chetty, Asst. Professor, Dept. of Botany, S V University, Tirupati.

Preparation of extract: The Extraction is needed to extract the desired chemical components from the plant materials for further separation and characterization. Prior to extraction process like pre-washing, drying of plant materials or freeze drying and grinding to obtain a homogenous sample are performed. Different solvent systems were used to extract the bioactive compound from natural products. Various methods like, sonification, continuous hot percolation (Soxhlet extraction), maceration, microwave assisted etc. were used for

extraction. Preliminary phytochemical screening was carried out to identify the presence of alkaloids, flavonoids, tannins, glycosides, proteins, and other constituents using standard qualitative tests. Thin Layer Chromatography (TLC) was performed for identification of phytoconstituents, and rutin content was estimated using UV spectrophotometry. Antioxidant activity was evaluated using the DPPH free radical scavenging assay, while antimicrobial activity was assessed using the agar well diffusion method against bacterial strains such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa. Polyherbal gel formulations were prepared using different gelling agents such as Carbopol, sodium CMC. The formulations were evaluated for physicochemical parameters including pH, viscosity, spreadability, homogeneity, stability, and in vitro drug release.

Results and Discussion

Table 1: Physicochemical evaluation

Evaluation	*Plumbago zeylanica L*	*Datura stromonium L*	*Argemone Mexicana L*
Color	Dark brown	Dark brown	Dark brown
Consistency	Solid	Solid	Semisolid
% yield	9.37%	12.20%	8.95%

Physicochemical evaluation of extracts: The colors of all extracts were dark brown, Plumbago zeylanica and Datura stramonium extracts are solid in nature while Argemone mexicana extract is semisolid. Percentage yields of the extracts are 9.37%, 12.20% and 8.95% respectively.

Table 2: Solubility of extracts

Solvent	*Plumbago zeylanica L*	*Datura stromonium L*	*Argemone Mexicana L*
Methanol	Soluble	Soluble	Soluble
Ethanol	Insoluble	Slightly soluble	Insoluble
n-hexene	Insoluble	Insoluble	Insoluble
Ethyl acetate	Insoluble	Insoluble	Insoluble
Chloroform	Insoluble	Soluble	Insoluble
Water	Insoluble	Insoluble	Soluble

Table 3: Preliminary phytochemical screening of different methanol plant extracts

Phytoconstituents	*Inference*			
	PZME	*DSME*	*AMME*	*PHME*
Triterpenoids	+	+	+	+
Tannins	+	+	+	+
Steroids and sterols	+	+	+	+
Saponins	+	+	+	+
Proteins	+	+	+	+
Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Carbohydrates	+	+	+	+
Alkaloids	+	+	+	+

+ = Components present

Phytochemical estimation of PZME, DSME and AMME revealed the presence of diverse phytoconstituents and showed that these plant extracts after being incorporated as polyherbal (PHME) does not have any impact on its phytocomposition and was found to possess all phytoconstituents as shown by other individual methanolic extracts.

Table 4: Preliminary phytochemical screening of different methanol: water (70:30) plant extracts:

Phytoconstituents	*Inference*			
	PZMW	*DSMW*	*AMMW*	*PHMW*
Triterpenoids	+	+	+	+
Tannins	+	+	+	+
Steroids and sterols	+	+	+	+
Saponins	+	+	+	+
Proteins	+	+	+	+
Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Carbohydrates	+	+	+	+
Alkaloids	+	+	+	+

+ = Components present

Phytochemical investigation of PZMW, DSMW and AMMW revealed the presence of diverse phytoconstituents. The screening showed that these plants extract after being incorporated as polyherbal (PHMW) does not have any impact on its phytocomposition and was found to possess all phytoconstituents as shown by other methanolic water extract

Table 5: Determination of Rf values

Sample extract	No. of spots	Rf value	Inference
Alkaloids (Cyclohexane: Chloroform: Diethyl amine 5:4:1)			
AMME	*1*	*0.511*	*Protropin derivatives*
DSME	*2*	*0.13*	*Scopolamine*
		0.48	*Scopoline derivatives*
PZME	*2*	*0.37*	*Plumbogin*
		0.23	*Napthaquinone derivatives*
Tannins (Chloroform: Ethyl acetate: Acetic acid 50:50:10)			
AMME	*3*	*0.48*	*Ferulic acid*
		0.57	*Cinnamic acid*
		0.71	*Cinnamic acid derivatives*
DSME	*4*	*0.76*	*Cinnamic acid derivatives*
		0.53	*Ferulic acid*
		0.25	*Paragallol*
		0.68	*Cinnamic acid derivatives*
PZME	*1*	*0.60*	*Cinnamic acid derivatives*
Phenol/Flavonoid (Toluene: Ethyl acetate: Formic acid 36:12:5)			
AMME	*3*	*0.56*	*Ferulic acid*
		0.77	*6-Hydroxy flavones*
		0.88	*Flavone*
DSME	*5*	*0.46*	*7-hydroxy flavone*
		0.56	*Ferulic acid*
		0.67	*6-Hydroxy flavones*
		0.70	*6-Hydroxy flavones derivatives*
		0.88	*Flavones*
PZME	*2*	*0.67*	*6-Hydroxy flavones*
		0.70	*6-Hydroxy flavones derivatives*

Table 6: *Synergistic antioxidant effect of polyherbal methanol extract (PHME)*

Sample	% inhibition at different concentration			
	2mg/ml	4mg/ml	6mg/ml	8mg/ml
AA	*63.84±1.263b*****	*67.59±0.304b****	*87.26±1.909b*****	*92.92±0.790b*****
PZME	*20.52±0.397a**** b*****	*56.77±0.447a**** b**	*57.82±0.599a**** b*****	*74.13±0.520a**** b*****
DSME	*17.09±1.064a**** b*****	*44.44±0.150a**** b*****	*47.77±1.611a**** b*****	*75.35±1.267a**** b*****
AMME	*13.77±0.728a**** b*****	*45.88±0.193a**** b*****	*61.93±0.220a**** b*****	*78.64±0.939a**** b**
PHME	*40.43±0.872a*****	*61.10±0.728a****	*71.78±1.599a*****	*82.79±1.718a*****

*AA: Ascorbic acid, PZME: Plumbago zeylanica linn., DSME: Datura stramonium l., AMMME: Argemone Mexicana linn. All values are represented as mean ± SEM, n = 3 for each group, Data were analyzed by two-way ANOVA, for each bacterial strain, followed by Dunnett's multiple comparisons test Multiple Comparisons Test, ****p< 0.0001 ***p< 0.001, **p< 0.01, *p< 0.1.* ***(a)*** *Signifies significant difference as compared to ascorbic acid (standard drug) and* ***(b)*** *denotes significant difference as compared to test drug PHME.*

Preparation of standard curve of Rutin

The plant extractives (both methanolic and macerated methanolic water extract) contains various constituents. The flavonoids are most active against microbial viability and are effective in tissue remodeling. Hence flavonoid content was estimated by taking rutin flavanoid as standard using Aluminum chloride colorimetric method. Standard curve was prepared separately by dissolving rutin in methanol followed by serial dilution in the concentration range from 1 – 50 µg/ml.

Rutin content of methanolic extract:

The Rutin content for PZME, AMME and DSME was found to be 3.55 ±1.52%, 10.30 ± 1.84% and 13.26 ± 1.65 % w/w respectively. Rutin content of macerated methanolic water extract. The Rutin content for PZMW, AMMW and DSMW was found to be 2.55 ±1.52%, 6.30 ± 1.84% and 8.26 ± 1.65 % w/w respectively. On the basis of the quantitative estimation of rutin content of the various methanolic and macerated (methanol: water) extract, it has been found that the methanolic extractives (PZME, DSME, AMME and PHME) had more flavonoid content. The bioactive have greater pharmacological significance as antioxidant, antimicrobial and tissue remodeling interventions, hence it was divided to explore the potential therapeutic efficacy of the different methanolic extracts and also to optimize its appropriate ratio for developing polyherbals.

Estimation of phytoconstituents by TLC:

The Rf values of samples PZME, DSME and AMME revealed the presence of alkaloids, tannins and phenols/ flavonoids. The values were matched with the standards and an inference was drawn (Table 5.5). The sample DSME revealed presence of tannins (4 spots) and flavonoids/phenolic compound (5 spots). Ferulic acid, 7-hydroxy flavone, 6-Hydroxy flavones and 6-Hydroxy flavones derivatives were inferred as phenolic and flavonoid compounds. In the same way, cinnamic acid derivatives, Ferulic acid and paragallol were inferred as tannins based on their Rf values. The (TLC) Thin layer chromatography was performed using standard methods (Harborne, 1998). All selected samples of PZME, DSME and AMME (2 mg/ml) were

dissolved in methanol. Solvent systems, developed by varying the concentration of mobile phases were used. The plates were visualized directly after drying and with the help of UV-TLC viewer. The Rf value of the different spots so obtained were calculated and these values were compared with the reference values from research articles like Svendsen AB, 1983; Sharma OP et al., 1998; Medić-Šarić M et al., 2004.

Determination of zone of inhibition of individual and polyherbal extract by agar well diffusion technique.

The antimicrobial spectrum showed that all the test samples were found to be effective against both gram positive and gram-negative strains in a concentration dependent manner. The zone of inhibition of the test samples when compared at each concentration level for individual strains, it was found that AMME exhibited antimicrobial efficacy with least zone of inhibition (ZI) against S. aureus, S. bacillus, E. coli and P. aeruginosa at 200 mg/ml. The sample PHME was found to be significantly more effective (Maximum ZI among all test samples) against S. aureus, S. bacillus, E. coli and P. aureginosa at maximum concentration level (200 mg/ml) which clearly showed the combinational effect of methanolic extracts. The results were showed antimicrobial efficacy in order viz. PHME > PZME > DSME > AMME. Ofloxacin shows its maximum antimicrobial efficacy as standard drug as compared to PHME under consideration. The results showed the synergistic effect of PHME in comparison to individual methanolic fractions against both gram positive and negative strains. The antimicrobial activity of the selected plant extracts and its polyherbal composition were consistent with the ethnopharmacological relevance.

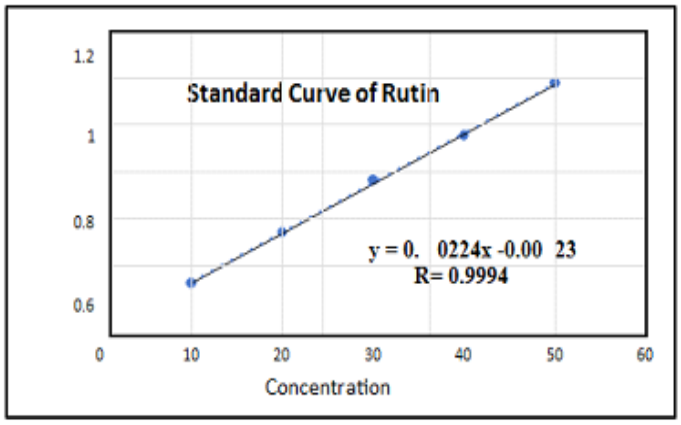

Figure 1: Standard curve of Rutin

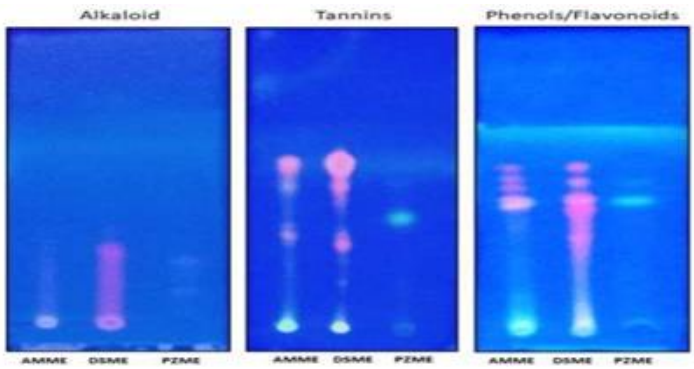

Figure 2: TLC Images of selected extracts

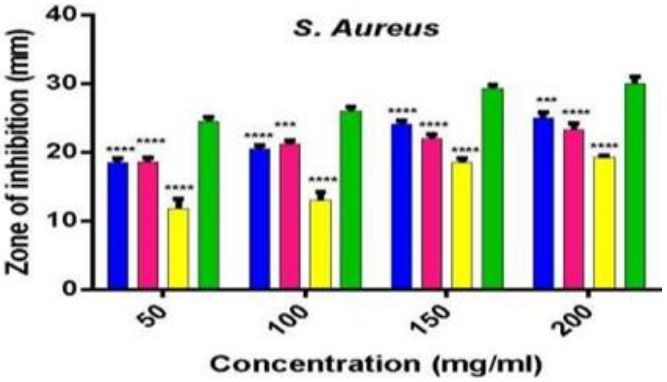

Figure 3: Antimicrobial spectrum of different plants extract and polyherbal against S.Aureus

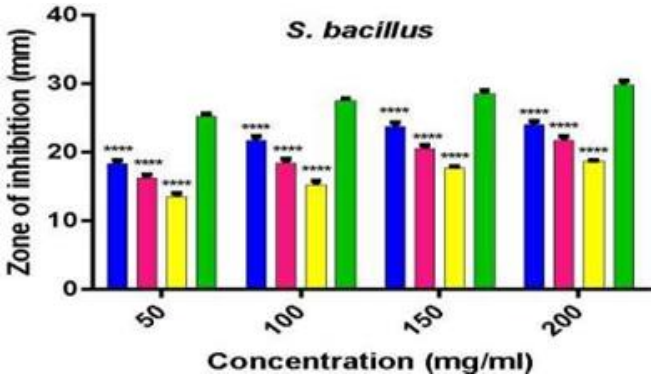

Figure 4: Antimicrobial spectrum of different plants extract and polyherbal against S. *bacillus*

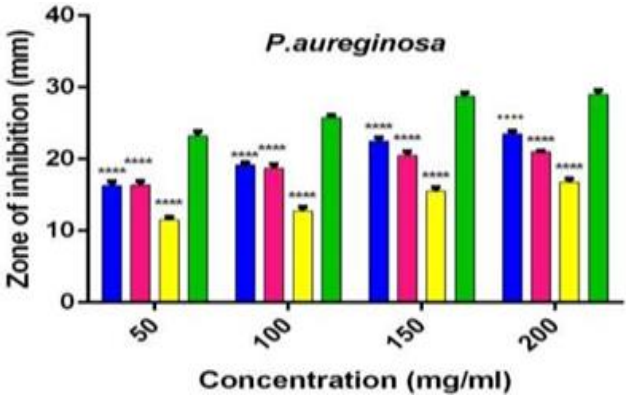

Figure 5: Antimicrobial spectrum of different plants extract and polyherbal against *P. aureginosa*

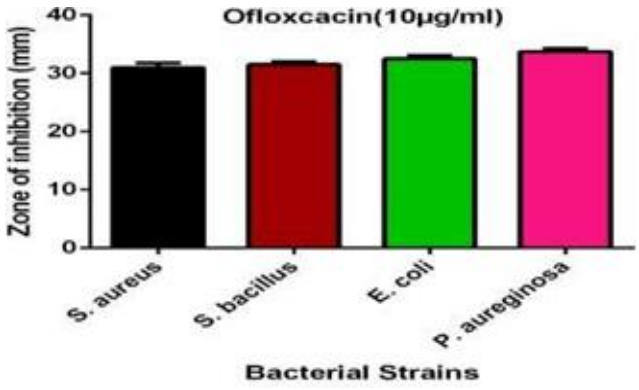

Figure 7: Antimicrobial activity of ofloxacin against different microbial strains at 10µg/ml

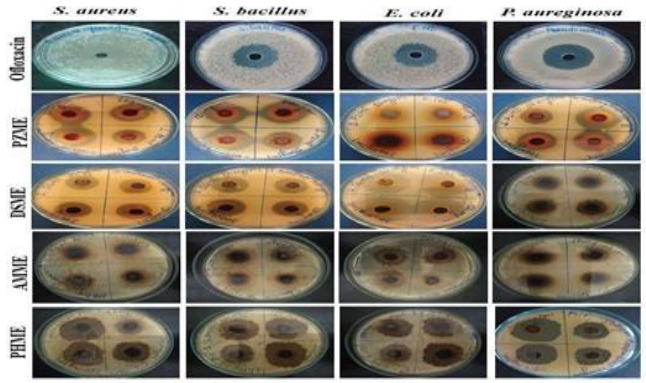

Figure 8: In-vitro culture plates (Agar cup plate method) of standard drug (ofloxacin) and Methanolic extracts (PZME, DSME, AMME and PHME) showing zone of inhibition (ZI) against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa at 50, 100, 150 and 200mg/ml concentrations.

Discussion

The extraction process yielded dark brown extracts with percentage yields of 9.37%, 12.20%, and 8.95% for Plumbago zeylanica, Datura stramonium, and Argemone mexicana, respectively. Phytochemical screening confirmed the presence of various bioactive constituents such as alkaloids, flavonoids, tannins, glycosides, saponins, and proteins, indicating their therapeutic potential. TLC analysis further confirmed the presence of compounds like plumbagin, scopolamine, ferulic acid, and flavonoid derivatives. Quantitative estimation of rutin content revealed higher flavonoid concentration in methanolic extracts, suggesting strong antioxidant potential.

The DPPH assay demonstrated concentration-dependent antioxidant activity, with the polyherbal extract showing maximum inhibition of 82.79%, indicating a synergistic effect of combined plant extracts. Antimicrobial studies revealed that all extracts exhibited activity against both gram-positive and gram-negative bacteria, with the polyherbal formulation showing the highest zone of inhibition, confirming enhanced efficacy due to synergistic interactions. The gel formulations exhibited acceptable physicochemical properties, including suitable pH, good homogeneity, and stability. Among the formulations, Carbopol-based gels showed superior stability and controlled drug release. Overall, the results indicate that the polyherbal formulation possesses significant antioxidant and antimicrobial activity, which contributes to enhanced wound healing potential.

Conclusion

The present study successfully developed and evaluated a polyherbal gel formulation using Plumbago zeylanica, Datura stramonium, and Argemone mexicana for wound healing activity. The phytochemical analysis confirmed the presence of diverse bioactive compounds responsible for therapeutic effects. The polyherbal extract demonstrated superior antioxidant and antimicrobial activity compared to individual extracts, indicating a synergistic effect. The formulated gels showed acceptable physicochemical properties, good stability, and sustained drug release. Among the formulations, Carbopol-based gel exhibited the best performance. The results suggest that the developed polyherbal gel is a promising alternative to conventional wound healing therapies. Further in vivo and clinical studies are recommended to validate its efficacy and safety for practical applications in wound management

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Conflict of Interests

The authors declare no conflict of interest

Ethics Approval: Not applicable

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AI Tool Declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

Data Availability

Data will be available on request

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